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RAT AND HUMAN DORSAL ROOT GANGLION ELECTRICAL AND OPTICAL SIGNALING USING THE *IN VITRO* CHIP-BASED HUMAN INVESTIGATIONAL PLATFORM (ICHIP)

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ABSTRACT

LLNL has demonstrated simultaneous fluorescence optical imaging and electrophysiological cell signal recording of dissociated human and rat dorsal root ganglia (DRG) nerve cells using a microfabricated *in vitro* chip-based human investigational platform (iCHIP) device. Both human and rat DRGs on the iCHIP devices after 16 days *in vitro* are physiologically relevant. Neurons have been cultured and shown viable in excess of 30 days *in vitro* on LLNL's iCHIP platform.

KEYWORDS

Human on a chip, thin film electrodes, hDRG, rDRG, Calcium imaging, extracellular action potential, microelectrodes, microelectrode arrays, neuron, *in vitro* electrophysiology

INTRODUCTION

Advances in modern medicine, drug discovery in particular, rely on the continued study of the human body to develop treatments for health problems ranging from high cholesterol and chronic joint pain to the effects of chemical exposure. However, the drug development cycle, from initial research and formulation to clinical trials and commercialization, can take years. Even after such lengthy study, about 90 percent of new drugs ultimately fail because they have unanticipated but serious side effects, many of which do not manifest in early studies conducted in animal models. Therefore, an integrated *in vitro* testing platform, comprised of cultured human cells from relevant tissues, would provide a testing modality to identify specific side effects in early testing phases and in an appropriate relevant model environment. To this end, we have demonstrated the effectiveness of a novel platform to sustain and interrogate isolated human dorsal root ganglia (DRG) nerve cells, which form part of the peripheral nervous system as a starting tissue for our integrated system. As envisioned, the complete iCHIP platform will contain tissue from the central nervous system, blood–brain barrier, stomach, liver, heart, kidney, brain, immune system, and lungs, effectively creating the tantalizing first steps towards a fully integrated human-on-a-chip. This technology has the potential to dramatically reduce the time needed to establish countermeasures against biological, chemical, and radiological agents and to bring new therapies to market.

This paper reports the successful recording of chemically-evoked electrophysiological and optical fluorescence signals from dissociated rat and human DRG cells seeded onto fabricated 4x4 microelectrode arrays (MEAs). A number of research groups have recorded signals from various types of animal model cells utilizing MEAs. For example, Rolston, *et al.* manufactured large array systems with supporting hardware to measure signals from both tissue slices and dissociated cell cultures [1]. Kolpas, *et al.* demonstrated fluorescence signaling of dissociated cells when challenged with chemical and therapeutic compounds [2]. However, this report is the first demonstration combining orthogonal detection modalities using both rat and human DRG cells. In this paper we present the first simultaneous optical and electrophysiological measurements.

DEVICE FABRICATION

Glass wafers were sputter coated with Ti/Pt as a metal trace/electrode layer. Positive photoresist was deposited and patterned using traditional photolithography. A metal etching system (ULVAC)

defined the metal electrodes and traces and was followed by a STS AOE polymer etch to remove the photoresist. Polyimide was deposited, imidized and etch-patterned through an aluminum mask. After removing the aluminum mask, the wafer was then coated with photoresist and diced to separate the devices. The protecting photoresist was removed with Acetone/IPA/water. Custom Omnetics neural connectors were bonded to the devices with silver epoxy and subsequently potted with a 2-part epoxy for mechanical strength. A polystyrene ring was attached with silicone adhesive to the device, encompassing the electrode array and defining the fluid well. Upon fabrication, each iCHIP device was quality assessed utilizing a Princeton Applied Research electrochemical system (PAR) to determine each electrode's viability with phosphate buffered solution. The same system was used to electroplate Pt onto the electrodes using a l00x linear sweep of -0.1 V to 0.1 V in an acidic Pt salt solution. The electroplated device was then measured by cyclic voltammetry over a linear sweep of -0.6 V to 0.8 V to determine the impedance and capacitance of each electrode.

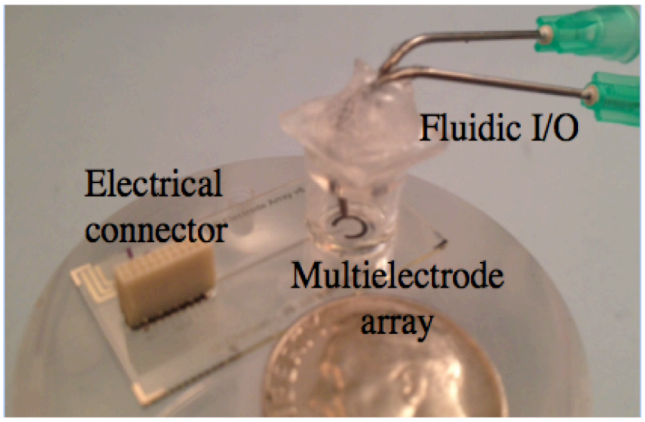

Figure 1: iCHIP (In Vitro Chip-Based Human Investigational Platform) with fluidic I/O

EXPERIMENT

Primary rat DRGs were isolated from 3-week-old animals. Human primary DRGs were obtained in collaboration with AnaBios Corporation. The fabricated MEA surfaces were treated with poly-D-lysine/laminin to enhance cell attachment and viability during the seeding procedure.

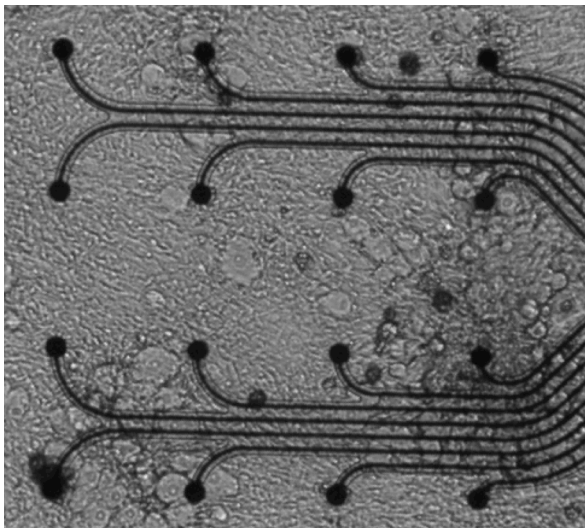

Figure 2: Bright field image of iCHIP device seeded with rat DRG cells and associated supporting glial cells

Chemical challenges and rinses (Hanks Balanced Salt Solution) were controlled by an automated FloPro fluidic handling system. This closed-loop, automated fluidic handling system provided precise control over challenge and rinse volumes (365ul and 450ul, respectively) and flow rates (25ul/sec). Figure 1 shows the fabricated iCHIP device with attached fluidic I/O cap. This cap allows for fluid introduction directly to the cell layer and is integrated with a thermocouple for in situ temperature measurements of the well fluid. Figure 2 shows a bright field image of an iCHIP device seeded with primary rat DRGs (and associated support glial cells) and the 16 active electrodes (counter thin film counter electrode not shown). We chose capsaicin as a chemical challenge material as it acts on DRG neurons to open cation-selective ion channels in the cell membrane. Fluorescence changes of the intracellular Ca+ indicator dye (Fluo8) from the 16 days *in vitro* human DRG cells when challenged with 500nM capsaicin are shown in Figure 3a. Figure 3b shows the recorded electrical response from the same human DRGs, when challenged with 500nM capsaicin. Importantly, rinsing the capsaicin from the device with 37°C buffer solution stopped the cell signaling, confirming that the cell responses were due to chemical interactions and not mechanical stimulation.

RESULTS/CONCLUSION

We have demonstrated the simultaneous collection of electrophysiology recording of extracellular neural action potentials and cellular Ca+ influx as a function of chemical stimulation in both human DRGs and rat DRGs. The ability to perform both electrophysiological and optical fluorescent measurements of these cellular responses has not been previously demonstrated. These measurements represent an important foundation for future experiments, whereby perturbation to these baseline measurements will elucidate neuron health as a function of chemical challenge. Our next steps include introducing other cell types to our system, and further integrating discrete devices into a single platform. Importantly, our ability to establish viable cultures of human neurons, maintain these cultures for greater than 30 days, and perform serial measurements, represents a significant step towards developing a human, multi-organ *in vitro* screening device. As such, LLNL's iCHIP platform will enable 'Human-on-a-Chip' response measurements of separated multiple cell types in an integrated system. This system has the potential to perform cellular drug reaction studies with a reduced need for animal protocol studies and can pave the way for more integrated human systematic response trials for drug interactions.

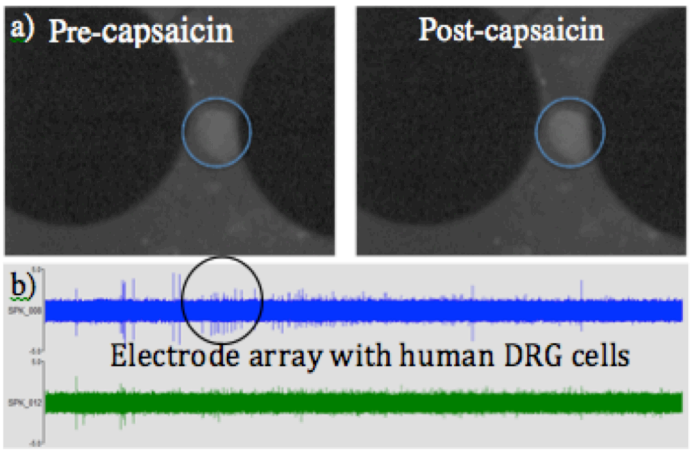

Figure 3: Measured signals from iCHIP seeded human DRG (16 DIV) signals when challenged with 500nM capsaicin a) Intracellular calcium dye fluorescence intensity changes b) Extracellular electrical signals measured by the iCHIP microelectrode array

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